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Duration of Infection and Antigen Display Have Minimal Influence on the Kinetics of the CD4+ T Cell Response to Listeria monocytogenes Infection

Gail A. Corbin* and John T. Harty2*†

The T cell response to infection consists of clonal expansion of effector cells, followed by contraction to memory levels. It was previously thought that the duration of infection determines the magnitude and kinetics of the T cell response. However, recent analysis revealed that transition between the expansion and contraction phases of the Ag-specific CD8+ T cell response is not affected by experimental manipulation in the duration of infection or Ag display. We studied whether the duration of infection and Ag display influenced the kinetics of the Ag-specific CD4+ T cell response to Listeria monocytogenes (LM) infection. We found that truncating infection and Ag display with antibiotic treatment as early as 24 h postinfection had minimal impact on the expansion or contraction of CD4+ T cells; however, the magnitudes of the Ag-specific CD4+ and CD8+ T cell responses were differentially affected by the timing of antibiotic treatment. Treatment of LM-infected mice with antibiotics at 24 h postinfection did not prevent generation of detectable CD4+ and CD8+ memory T cells at 28 days after infection, vigorous secondary expansion of these memory T cells, or protection against a subsequent LM challenge. These results demonstrate that events within the first few days of infection stimulate CD4+ and CD8+ T cell responses that are capable of carrying out the full program of expansion and contraction to functional memory, independently of prolonged infection or Ag display. The Journal of Immunology, 2004, 173: 5679–5687.

Listeria monocytogenes (LM) is a Gram-positive, facultative intracellular bacterium with the potential to cause severe disease in immunocompromised individuals. After uptake by phagocytic cells, LM use the virulence protein listeriolysin O (LLO) to escape from the phagosome into the host cell cytosol (1, 2). Bacterial proteins secreted into the host cell cytosol are degraded by host proteolytic machinery and presented by MHC class I molecules, inducing potent CD8+ T cell responses (3, 4). LM that fail to escape from the phagosome are destroyed, and bacterial proteins are processed and presented by MHC class II molecules to CD4+ T cells (5). Although both CD4+ and CD8+ T cells respond to LM infection, immunity to LM is mediated primarily by Ag-specific CD8+ T cells (6–10). However, recent data revealed the importance of CD4+ T cells for the generation of functional CD8+ T cell memory after LM infection (11, 12). There is also evidence that T cell help or CD40L is required for the differentiation of effector memory CD8+ T cells after LM infection (13).

The CD8+ T cell response to LM infection has been studied extensively (14). The peak in the number of Ag-specific CD8+ T cells in the spleen occurs between 7 and 9 days postinfection (15–18) and is followed by a rapid contraction (~90%) to memory cell numbers that can remain stable for the life of the host. Although there is much information relating to how naive and memory T cells are activated and maintained, the mechanism regulating the transition between expansion and contraction of Ag-specific T cells is largely unknown. In many acute infection systems, the onset of the T cell contraction phase correlates with pathogen clearance (19), suggesting that the duration of infection determines when Ag-specific CD8+ T cells transition between the expansion and contraction phases of the immune response. However, recent data revealed that ampicillin treatment early after infection resulted in earlier clearance of LM infection and shortened duration of Ag display, but did not shorten the interval between initial infection and the onset of the CD8+ T cell contraction phase (17). Similarly, the onset of CD8+ T cell contraction is largely unaffected in mice with prolonged Ag display as a result of chronic lymphocytic choriomeningitis virus infection (17, 20, 21). Thus, the durations of infection and Ag display do not determine the onset of Ag-specific CD8+ T cell contraction, suggesting that the contraction phase is programmed by early events after infection.

In contrast, much less is known about the CD4+ T cell response to LM infection. CD4+ T cells contribute to immunity by promoting dendritic cell (DC) activation (22), secreting inflammatory cytokines that activate the antimicrobial activities of macrophages, and providing help for the Ab response (23). In general, activation of both CD4+ and CD8+ T cell responses requires Ag and costimulation (24, 25), and both cell populations exhibit similar overall kinetics of expansion and contraction to memory (26). However, comparison of CD4+ and CD8+ T cell responses to infection have revealed differences that may reflect their differing roles in immunity. For instance, the kinetics of CD4+ and CD8+ T cell responses are synchronized, but over the life of the host, stable numbers of Ag-specific memory CD8+ T cells persist, whereas CD4+ T cell memory declines over time (26, 27). In addition,
CD8\(^+\) T cell memory, but not CD4\(^+\) T cell memory, is reduced after heterologous infection (28). Differences in CD4\(^+\) and CD8\(^+\) T cell memory maintenance may reflect different requirements for cytokines or other signals involved in the survival and proliferation of these cells. Reports show that IL-7 promotes memory CD4\(^+\) and CD8\(^+\) T cell survival (29–32), whereas IL-15 regulates memory CD8\(^+\), but not memory CD4\(^+\), T cell proliferation (33–37). Thus, there are both similarities and differences between the CD8\(^+\) and CD4\(^+\) T cell responses to infection.

It is currently unknown whether the kinetics of the CD4\(^+\) T cell response are dictated by the duration of infection and Ag display or are programmed early after infection, as seen with the CD8\(^+\) T cell response. To address this issue, we compared Ag-specific CD4\(^+\) and CD8\(^+\) T cell responses in C57BL/6 mice after infection with LM in control mice or those treated with antibiotic to truncate infection. We also examined the resulting memory T cells for the ability to carry out secondary CD4\(^+\) and CD8\(^+\) T cell responses and provide immunity to LM challenge.

Materials and Methods

Mice

C57BL/6 (B6) mice were obtained from the National Cancer Institute (Frederick, MD). B6.PL-Thy-1a (B6.PL) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained by brother-sister mating under specific pathogen-free conditions. Mice were initially used at 6–10 wk of age.

Bacteria and infection of mice

Recombinant LM expressing secreted OVA protein (rLM-OVA) (38) was provided by H. Shen (University of Pennsylvania School of Medicine, Philadelphia, PA). Bacteria were grown in tryptic soy broth with 50 \(\mu\)g/ml streptomycin and 50 \(\mu\)g/ml tetracycline, then diluted in 0.9% sodium chloride (Abbott Laboratories, Chicago, IL), and 10\(^4\) CFU of rLM-OVA was injected i.v. as previously described (39). The actual number of bacteria injected was confirmed by plate count. Some mice received ampicillin (2 mg/ml; Sigma-Aldrich, St. Louis, MO) in their drinking water beginning at various times postinfection; these mice were maintained on oral ampicillin for 7 days. In some experiments, mice were rechallenged with 5 \(\times\) 10\(^5\) CFU of rLM-OVA on the indicated days after primary infection. CFU per spleen were determined on various days after infection as previously described (40).

CD4\(^+\) T cell line

A CD4\(^+\) T cell line specific for LLO\(_{190-201}\) (41) was derived from B6 mice injected 30 days previously with 1 \(\times\) 10\(^5\) CFU of rLM-OVA. Briefly, 4 \(\times\) 10\(^5\) splenocytes from these mice were incubated in RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.2 mg/ml gentamicin, 50 \(\mu\)M 2-ME) at 37°C in 7% CO\(_2\) with 1.6 \(\times\) 10\(^6\) rat spleen cells and 50 mM 2-ME) at 37°C in 7% CO\(_2\) with 1.6 \(\mu\)g/ml (1 \(\mu\)M) LLO\(_{190-201}\) peptide. Subsequent restimulations were performed every 2 wk by combining 3 \(\times\) 10\(^5\) T cells with 5 \(\times\) 10\(^5\) irradiated (30 Gy) syngenic splenocytes that had been coated with 1.6 \(\mu\)g/ml LLO\(_{201-202}\), peptide for 30 min, followed by two washes in RP10. T cells and peptide-coated splenocytes were incubated in RP10 supplemented with 5% supernatant from Con A-stimulated splenic leukocytes/ml were labeled with a CD4-enriched splenocytes from rLM-OVA memory mice. Splenocyte cell suspensions were enriched for CD4\(^+\) T cells by negative selection (StemCell Technologies, Vancouver, Canada). Briefly 8 \(\times\) 10\(^5\) splenic leukocytes/ml were labeled with a CD4\(^+\) enrichment Ab mixture, followed by conjugation to magnetic beads. CD4\(^+\) T cells were obtained in the flow-through after magnetic separation (Miltenyi Biotec). Recovered cells were >95% CD4\(^+\) by flow cytometry.

Graded numbers of splenocytes obtained from mice between 1 and 3 days after primary rLM-OVA infection were cultured with 3 \(\times\) 10\(^4\) CD4\(^+\) enriched splenocytes from LM memory mice in round-bottom, 96-well microtiter plates in a final volume of 100 \(\mu\)l of RP10. Plates were incubated for 5 h at 37°C 7% CO\(_2\). After preincubation, cells were resuspended and transferred to rat anti-IFN-\(\gamma\) mAb-coated (1/100 dilution of RMMG-1 (BioSource International, Camarillo, CA) in PBS) wells for 15 min at 4°C. Cells were then washed three times in PBS-T, donkey anti-rabbit Ig conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA) was added according to the manufacturer’s instructions. After overnight incubation at 4°C, plates were developed with the Alkaline Phosphatase Substrate Kit III (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. The reaction was developed for 30 min at room temperature, and spots were counted using a dissecting microscope. The number of spots observed in wells containing infected splenocytes alone was subtracted from the number of spots in test wells (CD4\(^+\) enriched memory splenocytes plus infected splenocytes).

Direct ex vivo Ag detection (DEAD) assay

The DEAD assay was performed as previously described (17), except that an LLO\(_{190-201}\)-specific CD4\(^+\) T cell line was used to measure LLO\(_{190-201}\) Ag display. Briefly, 5 \(\times\) 10\(^5\) CFSE (Molecular Probes, Eugene, OR)-labeled, LLO\(_{190-201}\)-specific CD4\(^+\) T cells were mixed with 1 \(\times\) 10\(^5\) splenocytes obtained at various times from rLM-OVA-infected mice were incubated for 12 h, with brefeldin A (Sigma-Aldrich) added for the last 6 h of incubation. CFSE-labeled cells were then analyzed by ICS for IFN-\(\gamma\) or TNF. LLO\(_{190-201}\)-specific CD4\(^+\) T cells incubated with naïve splenocytes in the presence or the absence of synthetic LLO\(_{190-201}\) peptide served as positive and negative controls, respectively. Splenocytes prepared with or without DNase/collagenase treatment (44) were comprised of a similar fraction of CD11c\(^+\) cells (2.5–3.5%) and had a similar level of Ag, as detected by the DEAD assay (data not shown).

DC depletion

CD11c\(^+\) cells were depleted from splenocyte preparations by incubation with N418-FITC for 15 min at 4°C. After incubation, cells were washed and resuspended with 100 \(\mu\)l of microwebs (coated with anti-FITC Ab; Miltenyi Biotech, Auburn, CA) and 900 \(\mu\)l of PBS for 15 min at 4°C. Cells were passed over an LS MACS separation column (Miltenyi Biotech). Flow-through was collected, washed twice in RP10, and resuspended at 1 \(\times\) 10\(^6\) cells/ml. This depletion procedure was effective in reducing the percentage of CD11c to <0.8% of the total splenocytes.

ELISPOT-based Ag detection assay

Ag presentation by in vivo-infected splenocytes was also assessed with an ELISPOT-based assay as previously described (45), except that Ag was detected using CD4-enriched splenocytes from rLM-OVA memory mice. Splenocyte cell suspensions were enriched for CD4\(^+\) T cells by negative selection (StemCell Technologies, Vancouver, Canada). Briefly 8 \(\times\) 10\(^5\) splenic leukocytes/ml were labeled with a CD4\(^+\) enrichment Ab mixture, followed by conjugation to magnetic beads. CD4\(^+\) T cells were obtained in the flow-through after magnetic separation (Miltenyi Biotec). Recovered cells were >95% CD4\(^+\) by flow cytometry.

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Adoptive transfer experiments

B6.PL (Thy1.1) mice were infected with rLM-OVA. Seventy days after infection, 3 × 10^6 splenocytes were analyzed for CD4^+ LLO190–201-specific T cells and CD8^+ OVA257–264-specific T cells to determine the number of Ag-specific memory T cells present. Splenocytes were transferred i.v. into naive B6 (Thy1.2) mice to generate recipients containing the indicated number of Ag-specific memory cells (Thy1.1) in addition to endogenous naive precursors (Thy1.2). Recipient mice were infected 1 day later with 1 × 10^6 CFU of rLM-OVA. On the indicated days after challenge, recipient mice were euthanized and spleens were taken for analysis.

Results

Duration of infection and Ag display after LM infection

We examined whether the kinetics of the CD4^+ T cell response to LM infection were similarly programmed by early events after infection, as shown for the CD8^+ T cell response of BALB/c mice (17). However, no strong CD4^+ T cell epitopes are defined from LM-infected BALB/c mice. Thus, we could not use the BALB/c model to study concurrent CD4^+ T cell and CD8^+ T cell responses to infection. In contrast, LM infection of B6 mice stimulates a very strong, I-A^d-restricted, CD4^+ T cell response to aa 190–201 of the LLO protein (LLO190–201) (41). However, no strong MHC class Ia-restricted CD8^+ T cell epitopes are identified in LM-infected B6 mice. To circumvent this problem, we infected B6 mice with a recently described recombinant LM strain expressing hen OVA (rLM-OVA) as a secreted protein (38). Infection of B6 mice with rLM-OVA stimulates a vigorous expansion and the generation of memory CD4^+ T cells specific for LLO190–201 as well as a strong H-2K^b-restricted CD8^+ T cell response to the well-characterized OVA257–264 epitope (26). This system allowed us to directly compare CD4^+ T cells and CD8^+ T cell responses in the same host.

To investigate the role of duration of infection in both CD4^+ and CD8^+ T cell responses, rLM-OVA infection was attenuated in some mice with antibiotics, as previously described for analysis of the CD8^+ T cell response in BALB/c mice (17). Administration of ampicillin to mice via the drinking water beginning at 24 h postinfection resulted in an ~20-fold reduction in bacterial numbers 1 day later and complete clearance of bacteria by day 3 compared with clearance by day 7 in control infected mice (Fig. 1A). Thus, ampicillin treatment at 24 h postinfection results in rapid clearance of bacteria from infected mice and truncates the infection by 4 days compared with control infected mice.

We have previously shown that the number of LM in the spleen correlated with the amount of MHC class I Ag display detected using a direct ex vivo Ag detection assay (17). In these analyses, shortening the duration of infection with antibiotics also resulted in decreased MHC class I Ag display. Even though antibiotic treatment dramatically shortens the course of in vivo infection, it is possible that the level of MHC class II-bound LLO190–201 remains high. To determine whether MHC class II Ag display is also shortened by antibiotic treatment, we modified our DEAD assay (17) to use an LLO190–201-specific CD4^+ T cell line as a probe to measure relative LLO190–201 Ag amounts in the spleens of infected mice. We focused on the spleen because we did not detect bacteria or LLO190–201 Ag display in the brachial and cervical lymph nodes on days 1 or 3 after i.v. infection (data not shown). The frequency of CFSE-labeled LLO190–201-specific CD4^+ T cells that are stimulated in this assay to produce IFN-γ, as detected by ICS, is dependent on the dose of Ag (Fig. 1B). On day 2 postinfection, a substantially lower amount of the LLO190–201 Ag display was detected in spleen cells prepared from representative mice that had received ampicillin treatment 24 h postinfection, compared with the amount in mice that did not receive ampicillin (Fig. 1C). A kinetic analysis revealed that whereas LLO190–201 Ag display in control mice slowly declines between days 1–7 postinfection,
ampicillin treatment at 24 h caused a rapid reduction in the amount of LLO190–201 that fell below the level of detection on day 5, at least 2 days earlier than in control mice (Fig. 1D). Similar results were obtained using TNF production by the LLO190–201-specific CD4+ T cell line as the readout (data not shown).

The DEAD assay we used detects Ag display by any infected cell. However, it is likely that DC Ag display is the most meaningful in terms of stimulating naive T cell responses. To assess the contribution of DCs to MHC class II LLO190–201 display in the spleen, we depleted CD11c+ DCs by negative selection. We found that MHC class II LLO190–201 display on day 1 postinfection was significantly decreased after depletion of CD11c+ DC (Fig. 1E), suggesting that DCs account for at least some of the LLO190–201 Ag detectable in the spleen using the DEAD assay.

The LLO190–201-specific CD4+ T cell line used to probe LLO190–201 Ag display in the DEAD assay exhibits detectable background IFN-γ production (Fig. 1C; ~5% IFN-γ+CD4+ T cells) in the presence of naive splenocytes. To confirm the results of the DEAD assay, we performed an ELISPOT-based Ag detection assay (45) using LLO190–201-specific memory CD4+ T cells, in the form of CD4-enriched splenocytes from rLM-OVA immune mice, to probe Ag display on splenocytes from infected mice. The maximal number of spots (150/well) was detected at high input peptide amounts and decreased as the dose of peptide was reduced (Fig. 1F). Thus, the number of spots in the ELISPOT assay is saturable, but still can be used as a relative measure of Ag display. Similarly, the number of spots decreased as the input number of spleen cells from day 1 infected mice was reduced (Fig. 1G). On day 3, only a few spots were detected after culture of memory CD4+ T cells, with the highest input number of spleen cells from ampicillin-treated mice, whereas the same number of spleen cells from control mice stimulated a saturating response (~150 spots). Thus, the results of the ELISPOT-based detection assay were in agreement with the results obtained using the DEAD assay and showed that ampicillin treatment reduces the amount of LLO190–201 Ag display in the spleen early after infection. Together, these data suggest a direct relationship between the number of bacteria and the amount of LLO190–201 Ag display after rLM-OVA infection.

**CD4+ and CD8+ T cell responses after infection and antibiotic treatment**

To determine the effect of shortening the duration of infection and Ag display on the kinetics of the CD4+ and CD8+ T cell response in the same host, we measured LLO190–201-specific CD4+ and OVA257–264-specific CD8+ T cell responses on various days after rLM-OVA infection in control mice and in mice that received ampicillin treatment at 24 h postinfection (Fig. 2). The LLO190–201-specific CD4+ T cell response in mice infected with rLM-OVA and treated with ampicillin 24 h after infection was robust on day 7 postinfection and similar to the response in controls that did not receive ampicillin (Fig. 2, A and B). The peak of the LLO190–201-specific CD4+ T cell response was observed on day 8 in control mice and on day 7 in ampicillin-treated mice. However, the expansion in number between days 7 and 8 in control mice was <2-fold. Despite the apparent shift in the peak of the response, there was no significant difference in the rate or magnitude of contraction when the CD4+ T cell responses in ampicillin-treated and control mice were normalized to the respective peak days for each group (Fig. 2C). The numbers of LLO190–201-specific CD4+ T cells that remained after the contraction phase on day 28 were also not statistically different in ampicillin-treated and control mice. In general, ampicillin treatment had the same effect on the OVA257–264-specific CD8+ T cell response in the same mice (Fig. 2, D–F). These data show that ampicillin treatment, which substantially shortened the duration of infection and Ag display, had a minimal impact on the kinetics of the CD4+ T cell response after LM infection. Thus, the kinetics of the CD4+ and CD8+ T cell responses were not altered by ampicillin treatment.

![FIGURE 2](http://www.jimmunol.org/)  
Amoxicillin treatment after rLM-OVA infection minimally impacts the LLO190–201-specific CD4+ or OVA257–264-specific CD8+ T cell responses. C57BL/6 mice were infected with 1 × 10^9 CFU of rLM-OVA, and some of the mice were given 2 mg/ml ampicillin in their drinking water 24 h after infection. Ag-specific CD4+ and CD8+ T cell responses were measured by ICS for IFN-γ after incubation with or without LLO190–201 (A–C) or OVA257–264 (D–F) peptide, respectively. Numbers represent the percentage of LLO190–201-specific CD4+ T cells (A) or OVA257–264-specific CD8+ T cells (D) in splenocytes of representative mice from each group on day 7 after infection. The total number of Ag-specific CD4+ (B) or CD8+ (E) T cells per spleen was calculated from ICS. Data represent Ag-specific CD4+ (C) and CD8+ (F) T cell responses normalized to the number of Ag-specific T cells at the peak (day 7 or 8) of the response in each group. Data are the mean ± SD of five or six mice per time point and are representative of two independent experiments.
response in B6 mice are also largely independent of prolonged infection and Ag display.

Effect of differential antibiotic treatment on the magnitude of CD4\(^+\) T cell and CD8\(^+\) T cell responses

To determine the interval of infection needed for a full CD4\(^+\) and CD8\(^+\) T cell response on day 7, mice were inoculated with rLM-OVA and treated with ampicillin at 0, 24, or 48 h after infection. Bacterial clearance below the limit of detection (50 CFUs/spleen) was observed at 1–2 days after ampicillin treatment for all groups (Fig. 3A). In mice that received ampicillin at 0, 24, or 48 h postinfection, the peak of the CD4\(^+\) and CD8\(^+\) T cell responses was observed on day 7 compared with control mice, in which the peak of the T cell responses was observed on day 8 postinfection (data not shown). In mice that received ampicillin immediately after infection, the LLO\(_{190-201}\)-specific CD4\(^+\) T cell response on day 7 was 10-fold lower than the control response (Fig. 3B). Delaying ampicillin until 24 h after infection resulted in an LLO\(_{190-201}\)-specific T cell response that was significantly lower than the control response, albeit the reduction was only 2-fold. Similar levels of LLO\(_{190-201}\)-specific T cells relative to control were observed if ampicillin treatment was delayed until 48 h after infection. These data show that CD4\(^+\) T cells require 48 h of unmodified infection to generate the full response on day 7. As for the OVA\(_{257-264}\)-specific CD8\(^+\) T cell response, a slight reduction on day 7 was seen in mice given ampicillin immediately after infection, compared with control animals (Fig. 3D). Mice that received ampicillin at 0 or 24 h after infection had OVA\(_{257-264}\)-specific CD8\(^+\) T cell responses on day 7 that were similar to those in controls. These data suggest that CD8\(^+\) T cells only require 24 h of unmodified infection to generate the full response on day 7. Together, the results are consistent with the idea that a longer window of unmodified bacterial infection is required to activate or recruit the full complement of naive Ag-specific CD4\(^+\) T cells compared with CD8\(^+\) T cells after LM infection. Although the timing of antibiotic treatment did affect the magnitude of the response, it did not alter the overall kinetics of the CD4\(^+\) or CD8\(^+\) T cell response, because the expansion and contraction phases occurred with similar kinetics in all groups (Fig. 3, C and E).

Effect of antibiotic treatment on secondary T cell responses and protection after a subsequent infection

The data presented in this study show that ampicillin treatment after immunization with rLM-OVA has, at most, a modest impact on the number of CD4\(^+\) or CD8\(^+\) memory T cells on day 28 postinfection. However, the functionality of these cells may be altered by ampicillin treatment shortly after infection, as previous studies suggested that abridgement of LM infection with antibiotics resulted in decreased protection against a subsequent challenge (46). To determine whether ampicillin treatment after primary inoculation impacts the secondary T cell response, we challenged control immune or ampicillin-treated immune mice on day 28 postprimary infection with a high dose of rLM-OVA and examined the secondary Ag-specific CD4\(^+\) and CD8\(^+\) T cell responses. There was no difference in the kinetics or magnitude of secondary LLO\(_{190-201}\)-specific CD4\(^+\) T cell expansion in ampicillin-treated immune and control immune mice (Fig. 4A). Similarly, there was no difference in the kinetics or magnitude of secondary OVA\(_{257-264}\)-specific CD8\(^+\) T cell expansion in the same mice (Fig. 4B). These results show that ampicillin treatment after primary LM-OVA infection does not alter secondary expansion of memory CD4\(^+\) or CD8\(^+\) T cells in response to secondary rLM-OVA infection. Comparing

![Figure 3](http://www.jimmunol.org)
CD4+ and CD8+ secondary T cell responses, greater expansion from days 0–5 after rechallenge was observed for OVA257–264-specific CD8+ T cells (300-fold increase) than for LLO190–201-specific CD4+ T cells (60-fold increase; Fig. 4C). Nevertheless, both the CD4+ and CD8+ T cell secondary responses expanded faster and reached a higher number than that seen in the primary responses. Consistent with the high level of secondary T cell expansion in ampicillin-treated immune mice after rLM-OVA rechallenge, the level of bacteria recovered from the spleens of these mice was dramatically lower than that in LM-infected naive animals (Fig. 4C). On day 3 after rechallenge ampicillin-treated immune mice had a slightly higher bacterial load than control immune mice, which may be a consequence of the slightly reduced number of OVA257–264 CD8+ T cells. However, the reduction in bacterial numbers in ampicillin-treated immune mice compared with nonimmune mice was >10,000-fold, and both the ampicillin-treated immune mice and the control immune mice survived the infection and had no detectable bacteria in the spleen on day 5. In contrast, three of four naive mice challenged with rLM-OVA in this experiment died by day 5 postchallenge. Thus, we conclude that substantial protective immunity to lethal rLM-OVA rechallenge developed in B6 mice that received ampicillin treatment at 24 h after primary infection.

Kinetics of primary vs secondary CD4+ T cell response in the same host

We and others previously showed that contraction of the secondary CD8+ T cell response is prolonged compared with contraction of the primary CD8+ T cell response, independent of host environment or duration of infection (17, 47, 48). To investigate the kinetics of primary vs secondary CD4+ T cell responses and compare the kinetics of CD4+ and CD8+ T cell responses, day 70 memory T cells from rLM-OVA-immune B6.PL mice (Thy1.1+) were transferred into naive B6 recipients (Thy1.2+), and on 1 day post-transfer, recipient mice were infected with rLM-OVA. After infection, the endogenous primary response and secondary response of transferred LLO190–201-specific CD4+ T cells and OVA257–264-specific CD8+ T cells were measured by ICS (Fig. 5B). The endogenous primary CD4+ T cell response showed normal kinetics of expansion and contraction, such that ~10% of the cells present at the peak of the expansion remained on day 13 postinfection (Fig. 5C). The secondary response of the transferred memory LLO190–201-specific CD4+ T cells displayed delayed contraction compared with the primary response in the same animals, such that ~25% of the cells responding for the second time to infection were present on day 13. Consistent with previous results (17, 48), the contraction of the secondary CD8+ T cell response was also delayed compared with the primary response in the same host (Fig. 5D). These data show that contraction of both CD4+ and CD8+ secondary T cell responses are delayed compared with primary CD4+ and CD8+ T cell responses in the same host.

Ampicillin treatment at 24 h after infection does not alter the kinetics of the secondary CD8+ T cell response to LM rechallenge (17). To determine whether antibiotic treatment would alter the secondary CD4+ T cell response, day 70 memory T cells from B6.PL mice (Thy1.1+) were transferred into naive B6 recipients (Thy1.2+), and at 1 day post-transfer, recipient mice were infected with rLM-OVA. Recipient mice received ampicillin at 24 h postinoculation, which resulted in earlier bacterial clearance compared with nonampicillin-treated mice (Fig. 5A). The secondary response of the transferred memory LLO190–201-specific CD4+ T cells in mice that were infected and treated with ampicillin at 24 h postinfection also displayed delayed contraction compared with the primary response in the same animals (Fig. 5F). Consistent with previous findings (17), ampicillin treatment at 24 h postinfection also did not alter the secondary response kinetics of the transferred memory OVA257–264-specific CD8+ T cells (Fig. 5G). This shows that duration of infection does not alter the kinetics of either primary or secondary CD4+ or CD8+ T cell responses.

Discussion

After infection, CD4+ and CD8+ T cells become activated upon encounter with mature DCs displaying cognate Ag and costimulatory molecules. After activation, CD4+ and CD8+ T cells clonally expand and differentiate into effector T cells, that generally peak in number in the spleen and other organs between days 7 and 9 after LM infection (16–18, 26). After this peak, a contraction phase occurs, in which a majority of the Ag-specific CD4+ and CD8+ T cells die (26). Ag-specific CD4+ and CD8+ T cells that survive the contraction phase persist in the host as memory T cells.
The factors that determine the timing and magnitude of the contraction phase are unknown. The timing of contraction suggests that T cells sense the clearance of infection, then initiate the contraction phase, as survival cytokines become limiting. This model is consistent with the suggestion that a long period of Ag exposure, due to protracted clearance of infection, results in "fit" T cells that proliferate extensively and differentiate to long-lived memory T cells, whereas a short exposure to Ag results in abortive T cell proliferation, and tolerance. Both models predict that the duration of infection will determine the timing of the transition between contraction of Ag-specific T cells and the generation of memory. In contrast to this idea, we previously showed in BALB/c mice that ampicillin treatment after infection with attenuated LM does not alter the kinetics of the CD8<sup>+</sup>/H<sub>11001</sub>T cell response. This suggests that once the CD8<sup>+</sup>/H<sub>11001</sub>T cell response is initiated, it carries out the full program of expansion, contraction, and memory. In this study we used ampicillin treatment after LM infection of B6 mice to truncate the duration of infection and Ag display and determined the impact on Ag-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses and the generation of memory. Our results suggest that, similar to the CD8<sup>+</sup> T cell response, early events after infection also program the major features of the CD4<sup>+</sup> T cell response to infection.

We previously showed in BALB/c mice that ampicillin treatment at 24 h after infection with attenuated LM did not result in an earlier onset of contraction. In the current study we saw a slight reduction (2-fold) in the magnitude of expansion and a shift in the peak of both the CD4<sup>+</sup>/H<sub>11001</sub> and CD8<sup>+</sup>/H<sub>11001</sub>T cell responses to 1 day earlier in ampicillin-treated mice compared with control infected mice. This may reflect the use of a virulent LM strain in the current study, because the peak CD8<sup>+</sup>/H<sub>11001</sub>T cell response occurs 1–2 days later than that after attenuated LM infection. Alternatively, the difference may be due to the use of different mouse strains (B6 vs BALB/c) or the different T cell Ag specificities analyzed in the two studies. However, consistent with previous studies, we found that shortening LM infection with antibiotic treatment has only a minimal impact on the kinetics of CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses.
In previous studies it was shown that only 4-8 h of in vitro Ag exposure was required to stimulate multiple rounds of proliferation by transgenic CD8\(^+\) T cells (58), whereas ~20 h of Ag exposure was required to stimulate proliferation of transgenic CD4\(^+\) T cells (52, 59). Together, these results suggest that CD4\(^+\) T cells may require more time than CD8\(^+\) T cells for activation in response to Ag. Consistent with this idea, we show that a longer interval of unmodified infection is needed for full expansion on day 7 of the CD4\(^+\) T cell response compared with the CD8\(^+\) T cell response in the same host. The in vivo setting is more complex than the in vitro setting, because differences may exist in the kinetics of MHC class II and class I peptide/MHC complex generation or in the kinetics of APC encounter with T cells. However, we showed that the amount of class II Ag display, as detected in the DEAD assay, was highest on day 1 postinfection and then declined over time, suggesting that class II Ag processing and presentation occur rapidly after infection. Kaech et al. (60) have shown that whereas the level of LM infection controls the number of CD8\(^+\) T cell precursors recruited to respond and subsequently the magnitude of the response, all T cells that are recruited divide extensively. Thus, in our studies the requirement for a longer interval of unmodified infection to generate a maximal CD4\(^+\) T cell response may reflect different rates of recruitment between CD4\(^+\) and CD8\(^+\) T cells in vivo or could reflect a lower in vivo Ag-specific CD4\(^+\) T cell precursor frequency.

Secondary expansion of CD4\(^+\) T cells after rechallenge of ampicillin-treated immune and control immune groups was similar, as was secondary expansion of CD8\(^+\) T cells in the same mice, consistent with previous findings for memory CD8\(^+\) T cell responses in BALB/c mice (16). Ampicillin-treated immune and control immune mice also exhibited similar resistance to rLMM-OVA rechallenge. A previous study suggested that ampicillin treatment after LM infection resulted in loss of protective immunity against a subsequent challenge (46). This discrepancy may result from several differences in the experimental systems, such as the mouse and LM strains that were used. Additionally, we were able to measure memory and secondary Ag-specific CD4\(^+\) and CD8\(^+\) T cell responses in ampicillin-treated immune and control immune mice, whereas these analyses were not possible when the earlier study was performed. Other experimental differences were the duration of antibiotic treatment used to truncate LM infection. In contrast to the previous study, which showed that in our experimental conditions, prolonged infection is not required for a primary T cell response or protective immunity upon rechallenge. This result has implications for vaccine development, because it indicates that vaccination with live bacterial vectors and treatment with ampicillin allow the generation of protective immunity, with the added potential benefit of improved safety.

Studies comparing the kinetics of primary and secondary T cell responses in the same animal have shown that the contraction phase of the secondary CD8\(^+\) T cell response is prolonged compared with that of the primary response (17, 48, 61-64). Studies by Grayson et al. have demonstrated that memory T cells are more improved safety. Studies comparing the kinetics of primary and secondary T cell responses in the same animal have shown that the contraction phase of the secondary CD8\(^+\) T cell response is prolonged compared with that of the primary response (17, 48, 61-64). Studies by Grayson et al. have demonstrated that memory T cells are more

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